FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
APV31199	
U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	09/831816

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/CH98/00498	19 November 1998	

TITLE OF INVENTION

METHOD FOR PRODUCING L-PROLYL-L-M-SARCOLYSYL-L-P-FLUOROPHENYLALANINE AND DERIVATIVES THEREOF

APPLICANT(S) FOR DO/EO/US

Francesco MEHLEM
Pietro DE VITTORIO

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☒ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☒ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☐ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

Change of Name with Form PTO-1595
WO 00/31119 Cover Sheet

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/831816	PCT/CH98/00498	APV31199

24. The following fees are submitted:.		CALCULATIONS PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :		
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	**$1000.00**	
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	**$860.00**	
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$710.00**	
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$690.00**	
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	**$100.00**	
ENTER APPROPRIATE BASIC FEE AMOUNT =		**$860.00**
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		**$0.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	14 - 20 =	0	x $18.00	**$0.00**
Independent claims	1 - 3 =	0	x $80.00	**$0.00**
Multiple Dependent Claims **(check if applicable).**			☐	**$0.00**
TOTAL OF ABOVE CALCULATIONS =				**$860.00**
☒ Applicant claims small entity status. (See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				**$430.00**
SUBTOTAL =				**$430.00**
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				**$0.00**
TOTAL NATIONAL FEE =				**$430.00**
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				**$0.00**
TOTAL FEES ENCLOSED =				**$430.00**
			Amount to be: refunded	$
			charged	$

a. ☒ A check in the amount of **$430.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **19-4375** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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SIGNATURE

Anthony P. Venturino
NAME

31,674
REGISTRATION NUMBER

May 15, 2001
DATE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application

Francesco MEHLEM et al

Serial No.: (National Phase of PCT/CH98/00498)

Filed: May 15, 2001

For: METHOD FOR PRODUCING L-PROLYL-L-M-SARCOLYSYL-L-P-FLUOROPHENYLALANINE AND DERIVATIVES THEREOF

PRELIMINARY AMENDMENT

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

Prior to the calculation of the filing fee, please amend the above-identified application as follows:

IN THE CLAIMS

Please amend the claims as follows. A marked up set of the amended claims is attached (ATTACHMENT I).

1. (Amended) A method of producing at least one member of the group consisting of L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine, a lower alkyl ester thereof and acid addition salts thereof,

wherein L-p-fluorophenylalanine with a protected carboxyl group is caused to react with L-m-sarcolysine with a protected amino group and an activated carboxy group, L-m-sarcolysyl-L-p-fluorophenylalanine with a protected amino group and with a protected carboxy group being obtained, and subsequently the amino protection group is removed,

afterwards the obtained L-m-sarcolysyl-L-p-fluorophenylalanine with a protected carboxy group is caused to react with proline with a protected amino group and an activated carboxy group, L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine with a protected amino group being obtained, and the amino protection group being removed, and the lower alkyl ester group being optionally removed or converted into another ester group and/or the compound obtained being converted into an acid addition salt.

2. (Amended) The method according to claim 1, wherein the condensation is carried out with cooling in an anhydrous medium.

3. (Amended) The method according to claim 1, wherein the activated carboxy groups were activated through treatment with dicyclohexylcarbodiimid.

4. (Amended) The method according to claim 1, wherein the carboxy protection group of L-p-fluorophenylalanine is a lower alkyl ester group.

5. (Amended) The method according to claim 1, wherein the amino protection group of the L-m-sarcolysine is a carbobenzoxy group.

6. (Amended) The method according to claim 1, wherein the removal of the amino protection group of the L-m-sarcolysyl-L-p-fluorophenylalanine with a protected amino group is carried out through treatment with hydrogen bromide in glacial acetic acid.

7. (Amended) The method according to claim 1, wherein the removal of the amino protection group of the L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine with a protected amino group is carried out through reduction with hydrogen in the presence of palladium on carbon.

Please add new claims as follows.

--8. The method according to claim 2, wherein the condensation is carried out with cooling in chloroform.--

--9. The method according to claim 1, wherein the carboxy protection group of L-p-fluorophenylalanine is an ethyl ester group.--

--10. The method according to claim 2, wherein the activated carboxy groups were activated through treatment with dicyclohexylcarbodiimid.--

--11. The method according to claim 2, wherein the carboxy protection group of L-p-fluorophenylalanine is a lower alkyl ester group.--

--12. The method according to claim 2, wherein the amino protection group of the L-m-sarcolysine is a carbobenzoxy group.--

--13. The method according to claim 2, wherein the removal of the amino protection group of the L-m-sarcolysyl-L-p-fluorophenylalanine with a protected amino group is carried out through treatment with hydrogen bromide in glacial acetic acid.--

--14. The method according to claim 2, wherein the removal of the amino protection group of the L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine with a protected amino group is carried out through reduction with hydrogen in the presence of palladium on carbon.--

REMARKS

The claims have been amended to delete the multiple dependent claim status. No new matter is presented by the above amendments. Early and favorable consideration of this application is respectfully requested.

Respectfully submitted,

Date: May 15, 2001

By: [signature]

Anthony P. Venturino
Registration No. 31,674

APV/pgw
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ATTACHMENT I

Marked up set of claims

1. (Amended) A method of producing at least one member of the group consisting of L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine, a lower alkyl ester thereof and [and/or] acid addition salts thereof,

wherein L-p-fluorophenylalanine with a protected carboxyl group is caused to react with L-m-sarcolysine with a protected amino group and an activated carboxy group, L-m-sarcolysyl-L-p-fluorophenylalanine with a protected amino group and with a protected carboxy group being obtained, and subsequently the amino protection group is removed,

afterwards the obtained L-m-sarcolysyl-L-p-fluorophenylalanine with a protected carboxy group is caused to react with proline with a protected amino group and an activated carboxy group, L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine with a protected amino group being obtained, and the amino protection group being removed, and the lower alkyl ester group being optionally removed or converted into another ester group and/or the compound obtained being converted into an acid addition salt.

2. (Amended) The method according to claim 1, wherein the condensation is carried out with cooling in an anhydrous medium[, e.g. in chloroform].

3. (Amended) The method according to claim 1 [or 2], wherein the activated carboxy groups were activated through treatment with dicyclohexylcarbodiimid.

4. (Amended) The method according to [one of the claims 1 to 3] claim 1, wherein the carboxy protection group of L-p-fluorophenylalanine is a lower alkyl ester group[, preferably an ethyl ester group].

5. (Amended) The method according to [one of the claims 1 to 4] claim 1, wherein the amino protection group of the L-m-sarcolysine is a carbobenzoxy group.

6. (Amended) The method according to [one of the claims 1 to 5] claim 1, wherein the removal of the amino protection group of the L-m-sarcolysyl-L-p-fluorophenylalanine with a protected amino group is carried out through treatment with hydrogen bromide in glacial acetic acid.

7. (Amended) The method according to [one of the claims 1 to 6] claim 1, wherein the removal of the amino protection group of the L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine with a protected amino group [< is *carried out*>]] is carried out through reduction with hydrogen in the presence of palladium on carbon.

Method for Producing L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine and Derivatives Thereof

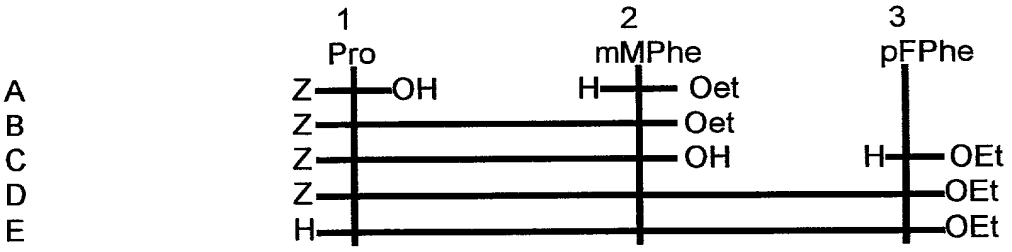
The present invention relates to a method for producing a pharmaceutically active peptide compound, which contains L-m-sarcolysine as the amino acid component. The active substance serves particularly for chemotherapy against cancers and is used especially for melanomas. In using a carrier substance on a cyclodextrin basis, the active substance is released delayed, which makes possible a sufficient bioavailability during a sufficiently long period of time.

A complex of six peptides containing m-L-sarcolysine has become known under the trade name "Peptichemio" (Insituto Sieroterapico Milanese S. Belfanti, Milan, Italy) for chemotherapy against cancer. It has been found that the activity of the individual peptides is different and that particularly one representative exhibits very high toxicity to melanoma cells. The peptides are a development which began with the product "Melphalan," i.e., 4-[bis(2-chloroethyl)]-amino-L-phenylalanine. It has been found that this product has a cytostatic effect and can be utilized both for myeloma and for melanoma therapy. For the further development of the active substance, derivatives of the product were prepared. This also resulted in the L-m-sarcolysine [= m-{di-2-chlorethyl)amino}-L-phenylalanine], which was further derived in that peptides were prepared which contained the modified amino acid as a component. A combination of the six oligopeptides L-seryl-L-p-fluorophenylalanyl-L-m-sarcolysyl ethyl ester; L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine ethyl ester; L-m-sarcolysyl-N-nitro-L-arginyl-L-norvaline ethyl ester; L-p-fluorophenylalanyl-L-m-sarcolysyl-L-asparagine ethyl ester; <*sic. L-p->* fluorophenylalanyl-glycyl-L-m-sarcolysyl-norvaline ethyl ester and L-m-sarcolysyl-L-arginyl-L-lysyl-L-m-sarcolysyl-L-histidine methyl ester formed the active principle of the antitumor agent "Peptichemio." Of the six peptides, the L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine (PSF) and its lower alkyl esters have proven particularly suitable.

It has been found that PSF showed considerably higher cytotoxicity compared with peptichemio itself (R. Levenson, et al., Radiumhemmet, Karolinska Hospital, Stockholm, Sweden, Eur. J. Cancer Clin. Oncol.; 23: 6, 783-788, 1987). According to these studies, it has been found that the peptide L-propyl-m-sarcolysyl-L-p-fluorophenylalanine (PSF) was 35 times and 28 times, respectively, more toxic to RPMI 8322 melanoma cells than melphalan and m-sarcolysine, respectively. Similar differences between the active substances have also been found for other melanoma cell lines.

It is the object of the present invention to make available a method of producing PSF that makes possible an economical and safe production of the active substance.

Production of such a compound is described in the printed publications BE-A-775775 and US-A-3 814 746. The described production takes place according to the following schema 1:



Pro = proline
mMPhe = m-[di(2-chlorethyl)]-amino-L-phenylalanine
pFPhe = p-fluoro-L-phenylalanine
Z = benzyloxycarbonyl

The above schema shows, in step A, the condensation of the N-carbobenzoxy-L-proline with the ethyl ester of m-[di-(2-chlorethyl)-amino]-L-phenylalanine, the corresponding protected peptide resulting, as is shown in step B in schema 1.

In step C, the N-carbobenzoxy-L-prolyl-m-[di-(2-chlorethyl)-amino]-L-phenylalanine is obtained from the N-carbobenzoxy-L-prolyl-m-[di-(2-chlorethyl)-amino]-L-phenylalanine ethyl ester, and subsequently the condensation of this compound is carried out with p-fluoro-L-phenylalanine ethyl ester, while in step D of the schema 1 the carbobenzoxy-L-prolyl-m-[di-(2-chlorethyl)amino]-L-phenylalanine-p-chloro-L-phenylalanine ethyl ester is obtained.

The protection group is eliminated, whereby one arrives at step E of schema 1, the end product being the L-prolyl-m-[di-(2-chlorethyl)amino]-L-alanine-p-fluoro-L-phenylalanine ethyl ester.

The reaction conditions are such as are used generally with peptide syntheses. With the above method, the end product is obtained with a yield of 30 %, with respect to the starting product m-[di-(2-chlorethyl)amino]-L-phenylalanine ethyl ester, it being necessary to carry out the purification of at least one intermediate product through column chromatography on silica gel.

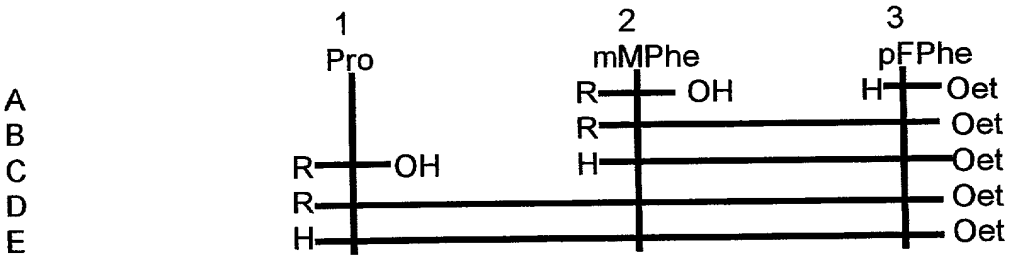
The method can actually be applied industrially, but it is relatively complicated and leads to a rather insufficient yield.

If one takes into consideration the properties of the end product PSF-hydrochloride, the realization of another method of production, which can easily be applied industrially and which results in a better yield than that of the state of the art, is then an extraordinarily important and interesting object of the present invention.

It has been found that the method for producing PSF according to the invention, which uses another reaction sequence, is superior to the state-of-the-art method.

The subject matter of the present invention is thus the method defined in claim 1 for producing L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine and esters and/or salts thereof.

The method according to the invention takes place according to the following schema 2:



Pro = proline
mMPhe = m-[di(2-chlorethyl)]-amino-L-phenylalanine (=L-m-sarcolysine)
pFPhe = p-fluoro-L-phenylalanine
R = benzyloxycarbonyl, t-butoxycarbonyl (BOC) or 9-fluorenylmethyoxycarbonyl (Fmoc)

The method comprises the following process steps, which are indicated in schema 2 above:

a) condensation of R-m-[di-(2-chlorethyl)amino]-L-phenylalanine with p-fluoro-L-phenylalanine ethyl ester, whereby R-m-[di-2-chlorethyl)amino]-L-phenylalanyl-p-fluoro-L-phenylalanine ethyl ester is obtained;

b) Removal of the protection group R ;

c) condensation of the product obtained in step b) with R-L-proline, R-L-prolyl-m-[di-(2-chlorethyl)amino]-L-phenylalanyl-p-fluoro-L-phenylalanine ethyl ester being obtained;

d) Removal of the protection group R and synthesis of the hydrochloride; R can be benzyloxycarbonyl, t-butyloxicarbonyl or 9-fluorenylmethoxycabonyl. R is preferably a benzyloxycarbonyl group.

The method according to the present invention shows a total yield of 50 % with respect to the starting product R-m-[di-(2-chlorethyl)amino]-L-phenylalanine.

The method according to the present invention has a great advantage in carrying out the synthesis of the end product since crystalline intermediate products are obtained which can be purified extraordinarily easily through crystallization.

The features and the advantages of the method according to the invention will be explained, for better comprehension, in the following description. The tripeptide, produced according to the inventive method, is produced according to schema 2 above. Therein R is a benzyloxicarbonyl or a t-butoxycarbonyl group (BOC) or a 9-fluorenylmethoxycarbonyl group (Fmoc).

As follows from schema 2, the method calls for, in step A, the condensation of R-m-[di-2-chlorethyl)amino]-L-phenylalanine with the ethyl ester of the p-fluorophenylalanine, the corresponding protected tripeptide being obtained in step B.

In step C, the benzyloxycarbonyl group is removed, and through a condensation of the R-L-proline with m-[di-(2-chlorethyl)amino]-L-phenylalanyl-p-fluoro-L-phenylalanine ethyl ester, the R-L-prolyl-m-[di-2-chlorethyl)amino]-L-phenylalanyl-p-fluoro-L-phenylalanine ethyl ester is obtained in step D of schema 2.

The protection group R is removed in step E of schema 2, the end product being L-prolyl-m-[di-(2-chlorethyl)amino]-phenylalanyl-p-fluoro-phenylalanine ethyl ester.

The reaction conditions are such as are generally standard with peptide synthesis.

The peptide L-prolyl-m-sarcolysyl-L-p-fluorophenylalanin (PSF) is produced preferably in the form of hydrochlorides or hydrobromides.

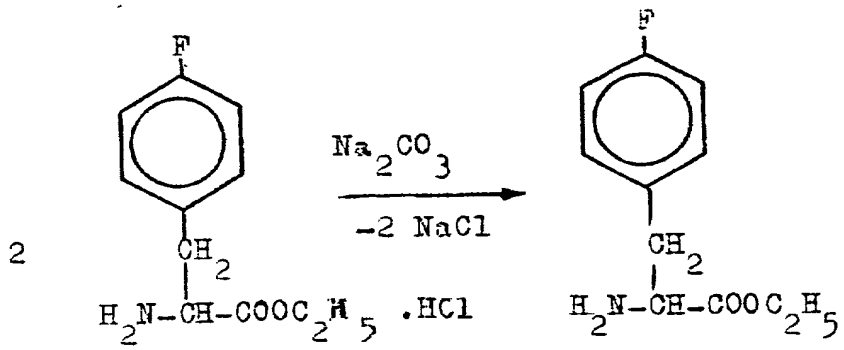
The following example serves the purpose of elucidation of the present invention.

Example:

Synthesis of L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine ethyl ester hydrochloride

a) N-carbobenzoxy-L-m-sarcolysyl-L-p-fluorophenylalanine ethyl ester

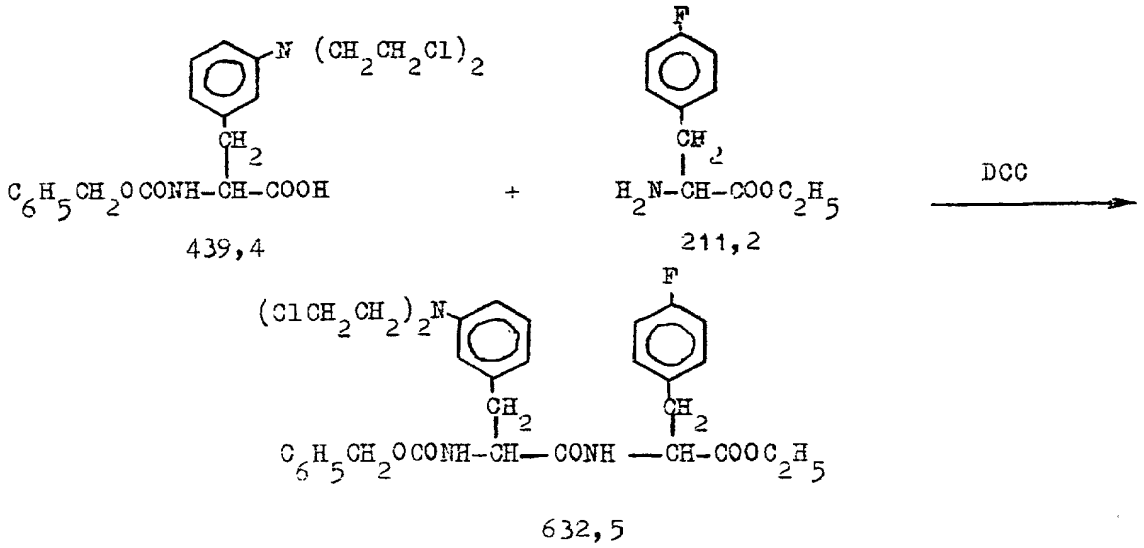
52.5 g of L-p-fluorophenylalanine ethyl ester hydrochloride are treated with 75 ml of Na_2CO_3 (sodium carbonate) saturated solution and 150 ml of $CHCl_3$. The mixture is shaken out, and the organic phase is separated and saved. The aqueous phase is shaken out a second time with 75 ml of $CHCl_3$. The combined chloroform extracts are mixed and washed once with water, and then separated from the aqueous phase and dried on anhydrous Na_2SO_4. The concentration of amino acid ester is determined by a titration with $HClO_4$ (perchloric acid). The yield corresponds approximately to the theoretical value; it is at 98%.



286.5 ml of a chloroform solution containing 0.1905 moles of L-p-fluorophenylalanine ethyl ester are reacted with 83.7 g (0.1905 moles) of N-cbzo-L-m-sarcolysine. The solution is cooled on an ice bath.

Added to the cooled solution with stirring are 41.25 g (0.200 moles of dicyclohexyl carbodiimide - DCC) and 60 ml of chloroform, the solution being

constantly stirred with simultaneous cooling for 30 min. The mixture may possibly solidify into a solid mass. In this case, the mass is made liquid again through addition of 150 ml of chloroform, it being stirred with slight warming. In this way, dissolving of the precipitated product is accelerated. The reaction is ended 2 hrs after addition of the DDC. The end of reaction is established by TLC checking (thin-layer chromatography; silica gel G layer, solvent: chloroform + acetone 9:1, manifestation by spraying with dilute, acid $KMnO_4$ solution). The precipitated dicyclohexyl urea is separated by filtration. The solution is washed first with little water, then with saturated Na_2CO_3 solution. The chloroform solution is shaken out once more with water and then dried with Na2SO4. The solvent is evaporated in vacuo and removed. After drying, 140.25 g of slightly yellowish-colored product is obtained (yield 98.3%). The substance produced has a melting point of 123-124.5°C and is chromatographically homogeneous. Through crystallization of 4.5 g of substance from 37.5 ml ethyl alcohol, 3.75 g of a lighter product are produced with a melting point of 125-126°C. α_D^{20}:27.7 (c = 2, $CHCl_3$).

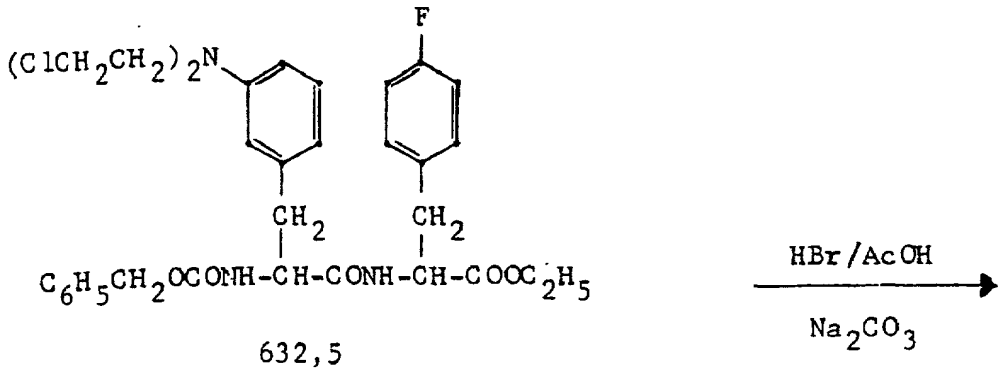


Analysis for $C_{32}H_{36}Cl_2FN_3O_5$

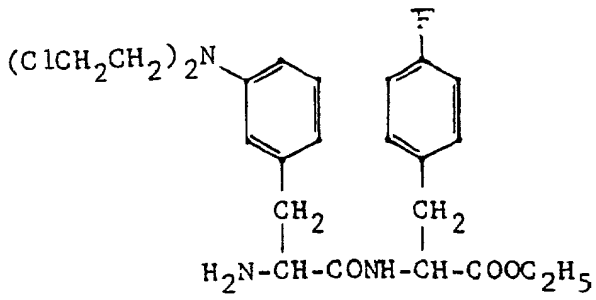
N% = 6.67 (6.66 calculated

Cl% = 11.5 (calculated = 11.2)

b) L-m-sarcolysyl-L-p-fluorophenylalanine ethyl ester



632,5



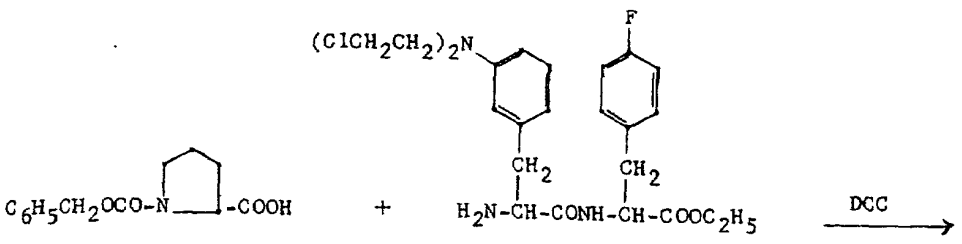
498,4

With exclusion of atmospheric humidity, 600 ml of HBr in glacial acetic acid (33%) are added with slow stirring to 390 g (0.616 moles) of die[?] M <*sic. ~~die[?] M~~ N->* -carbobenzoxy-L-m-sarcolysyl-L-p-fluorophenylalanine ethyl ester. Dissolving and cessation of the CO_2 development takes place after 40 minutes. It is allowed to stand for a further 20 minutes with stirring and diluted with approx. 400 ml of ether. The whole is poured into 5 lt of ether which is kept under constant stirring, is decanted, and the precipitated oil is washed twice with 2 lt of ether with decanting. The oil is treated with 4 lt of water with stirring, and a solid is obtained, which is collected after approx. 30 min. by filtration, and is completely washed with a total of 1500 ml of water and 500 ml of ether. The bromohydrate thus obtained is suspended in 2 lt of ethyl acetate and treated with stirring with 450 ml of saturated sodium carbonate solution, thus until the

solution is alkaline. After dissolving has taken place, filtration is carried out on the suction filter in order to remove the suspended dicyclohexyl urea (very little). In a separating funnel, the organic layer is separated from the aqueous phase, and the aqueous phase is extracted with a further 500 ml of ethyl acetate. The purified extracts are washed with 300 ml of water, Na_2CO_4 dried, and treated with norite. Filtration is carried out, and the filtrate is dried in vacuo (40°C). The residue is taken up even before it becomes firm in 500 to 1000 ml of ether. During the night, a white product is precipitated from the solution obtained. Yield: 247 g (80.4%) Melting point 100-102°C.

α_D^{20} = -7.5° (c=2, chloroform)
TLC ($BuOH/AcOH/H_2O$ 65:15:25; $KMnO_4$ diluted):
one band, Rf = 0,74
analysis for $C_{24}H_{30}Cl_2FN_3O_3$
N% = 8.34 (8.43 calculated)
Cl% = 14.1 (14.2 calculated)

c) N-carbobenzoxy-L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine ethyl ester



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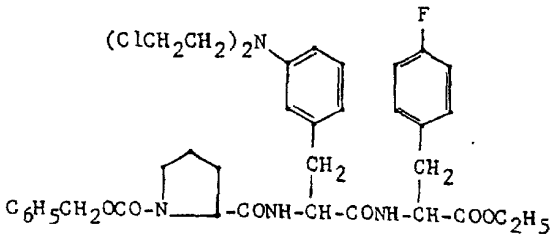

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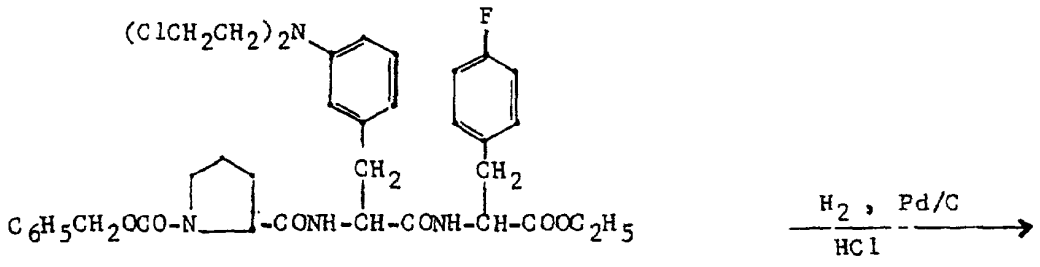
A mixture of 249 g (0.5 moles) of L-m-sarcolysyl-L-p-fluorophenylalanine ethyl ester, 125 g (0.5 moles) of N-cbzo-L-proline, and 109 g (0.525 moles) DCC in 3000 ml of chloroform is allowed to stand for 30 minutes with stirring, with external cooling for a further 90 minutes at room temperature (TLC, silica gel G, Chf/Me_2CO 9:1; or with BuOH/AcOH/H_2O 65:15:25; $KMnO_4$, diluted, acid). After removal of the dicyclohexyl urea by filtration, the solvent is evaporated off in vacuo, and the residue, still in liquid state, is poured into 800 ml of ether. From the solution obtained, the product precipitates slowly out, which is collected on a filter. Yield 290 g (78.5%). Melting point = 148-150°C, α_D^{20} = -42.4° (c=2; chloroform)

Analysis for $C_{37}H_{43}FCl_2N_4O_6$
N% = 7.78% (7.68 calculated)
Cl% = 9.6 (9.7 calculated)

d) L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine ethyl ester hydrochloride



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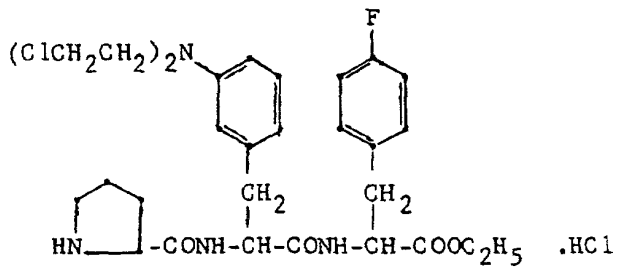

632

A mixture of 157.5 (0.261 moles) N-carbobenzoxy-L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine ethyl ester and 30 g of palladium on 5% carbon is suspended under a stream of nitrogen in 15 ml of glacial acetic acid and 1750 ml of methanol. The reaction mixture is kept stirred and is reduced under a stream of hydrogen. After termination of the CO_2 development (after 4-5 hours), a TLC chromatography check is carried out (silica gel G), elution taking place with chloroform acetone 9:1 and making visible with dilute $KMnO_4$.

After removal of the catalyst by filtration, the filtrate is acidified with concentrated ethanolic HCl in a stoichiometric amount or a little more. The white, crystalline precipitate which slowly forms is collected on a filter and washed with ethanol or with ether: 85 g. The filtrate is concentrated practically to dryness, and the residue is recrystallized from ethanol: 25 g. Complete yield: 110 g (80.5%); melting point 122-124°C (modification of the aggregate state)

α_D^{20} = 13.0° ± 0.5 (c=2; MeOH)

TLC (silica gel G; BuOh/AcOH/H_2O 65:15:25; $KMnO_4$ diluted: one band Rf = 0.54.

Analysis for $C_{29}H_{38}Cl_3FN_4O_4$

N % = 8.93% (8.86 calculated)

Cl % = 16.7% (16.8 calculated)

Cl-% = 5.65% (5.6 calculated)

Claims

1. A method of producing L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine, a lower alkyl ester and/or acid addition salts thereof, wherein L-p-fluorophenylalanine with a protected carboxyl group is caused to react with L-m-sarcolysine with a protected amino group and an activated carboxy group, L-m-sarcolysyl-L-p-fluorophenylalanine with a protected amino group and with a protected carboxy group being obtained, and subsequently the amino protection group is removed, afterwards the obtained L-m-sarcolysyl-L-p-fluorophenylalanine with a protected carboxy group is caused to react with proline with a protected amino group and an activated carboxy group, L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine with a protected amino group being obtained, and the amino protection group being removed, and the lower alkyl ester group being optionally removed or converted into another ester group and/or the compound obtained being converted into an acid addition salt.

2. The method according to claim 1, wherein the condensation is carried out with cooling in an anhydrous medium, e.g. in chloroform.

3. The method according to claim 1 or 2, wherein the activated carboxy groups were activated through treatment with dicyclohexylcarbodiimid.

4. The method according to one of the claims 1 to 3, wherein the carboxy protection group of L-p-fluorophenylalanine is a lower alkyl ester group, preferably an ethyl ester group.

5. The method according to one of the claims 1 to 4, wherein the amino protection group of the L-m-sarcolysine is a carbobenzoxy group.

6. The method according to one of the claims 1 to 5, wherein the removal of the amino protection group of the L-m-sarcolysyl-L-p-fluorophenylalanine with a protected amino group is carried out through treatment with hydrogen bromide in glacial acetic acid.

7. The method according to one of the claims 1 to 6, wherein the removal of the amino protection group of the L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine with a protected amino group < *is carried out*> through reduction with hydrogen in the presence of palladium on carbon.

Abstract

L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine, lower alkyl esters and/or acid addition salts thereof are produced. For this purpose, L-p-fluorophenylalanine with a protected carboxyl group is caused to react with L-m-sarcolysine with a protected amino group preferably with cooling in an anhydrous medium in the presence of dicyclohexylcarbodiimid, L-m-sarcolysyl-L-p-fluorophenylalanine with a protected amino group and a protected carboxyl group being obtained. Then the amino protection group is removed, with formation of L-m-sarcolysyl-L-p-fluorophenylalanine with a protected carboxyl group. The obtained product is caused to react with proline with a protected amino group in the presence of dicyclohexylcarbodiimid. L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine with a protected amino group is obtained. Finally, the amino protection group is removed, and optionally the lower alkyl ester group is removed and/or the obtained compound is converted into an acid addition salt.

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status (patented, pending, abandoned)
Application Serial No.	Filing Date	Status (patented, pending, abandoned)

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith; Stevens, Davis, Miller & Mosher, L.L.P.; Anthony P. Venturino, Reg. No. 31,674; James E. Ledbetter, Reg. No. 28,732; and Thomas P. Pavelko, Reg. No. 31,689. Direct all telephone calls to telephone no. (202) 785-0100 and faxes to (202) 202-408-5200.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of Sole, First Inventor	Inventor's Signature	Date
Residence:		Citizenship
Post Office Address:		

#3

COMBINED DECLARATION AND POWER OF ATTORNEY FOR UTILITY PATENT APPLICATION (Includes PCT)	Attorney Docket No.

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; that

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Method for Producing L-prolyl-L-m-sarcolysyl-L-p-fluorophenylalanine and Derivatives Thereof

the specification of which (check one)

[ ] is attached hereto.

[ ] was filed on ___ as Application Serial No. ____

and was amended on _____. (if applicable)

[X] was filed as PCT international application no. PCT/CH 98/00498

on 19.11.1998, and was amended under PCT Article 19

on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I do not know and do not believe the claimed invention was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to this application, that the same was not in public use or on sale in the United States of America more than one year prior to this application, that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months prior to this application.

I hereby claim foreign priority benefits under Title 35, United States Code §119 of any foreign application(s) and United States provisional applications for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application(s) on which priority is claimed:

Prior Foreign and U.S. Provisional Application(s)			Priority Claimed	
---	---	---	[ ]	[ ]
(Number)	(Country)	Day/Month/Year Filed	Yes	No
			[ ]	[ ]
(Number)	(Country)	Day/Month/Year Filed	Yes	No

Full Name of ~~Second~~, Joint Inventor First	Inventor's Signature	Date
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